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Notes on identity of *Picrorhiza* - a high value threatened medicinal plant group of western Himalaya

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पश्चिमी हिमालय के संकटग्रस्त उच्च मूल्य वाले औषधीय पादप समूह – *पिकोराइजा* की वर्गिकी पहचान पर टिप्पणी

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सारांश

प्रस्तुत शोध पत्र में *पिकोराइजा* रॉयली (विरोनिऐसी) वंश की तीन जातियों की वर्गिकी, पारिस्थितिक, जैवरासायनिक समीक्षा की गई है। इस शोध का प्रमुख उद्देश्य इन तीनों जातियों में विभेदन कर हर्बल उद्योग में इनके उच्च पैमाने पर होने वाली मिलावट की रोकथाम एवं आयुर्वेदिक चिकित्सा में सही जाति के उपयोग को बढ़ावा देना है।

ABSTRACT

A review of three species of genus *Picrorhiza* Royle (Veroniceae) presented here in view of the taxonomy, ecology and phytochemistry. The prime objective of the paper is to differentiate these three species to avoid high scale adulterants in herbal industry and to use correct species for the most efficacy in the Ayurvedic system of medicine.

Keywords: *Picrorhiza tungnathii*, Kutki, Himalaya, Drug Validation, Ayurveda

INTRODUCTION

The genus *Picrorhiza* Royle formerly belonging to the tribe Veroniceae of Family Scrophulariaceae, is now placed in Plantaginaceae (Albach & al., 2004a, 2004b). The genus (as *P. kurrooa*) appeared for the first time on a drawing published by Royle on August 24, 1835 in his Illustrations of the botany. The generic name was derived from the bitter root (picros means bitter, rhiza means root) used in native medicines (Royle, 1836); while the specific name is derived from Karu, the Punjabi name of the plant, which means bitter as well (Coventry 1927) Bentham (1835) described the genus and the species as (*P. kurroa*) in his Scrophularineae Indicae, which was published in November 17, 1835. The genus *Picrorhiza* was considered to be monotypic, until Pennell (1943) described *P. scrophulariiflora* from a distinct eastern Himalayan population (the 'shorter-stamen' form) in his revision of Himalayan Scrophulariaceae. Later, Hong (1984) observed that, in addition to important differences in the floral structure, these two species also differ in palynological characters of *P. kurrooa* Royle are

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of the *Scrofella* –type while those of *P. scrophulariaeflora* Pannell are of *Paederota* –type (Hong & al., 1998). Based on these Palynological and morphological characters, he proposed the new genus *Neopicrorhiza* Hong. Miller (2000) described third species, *N. minima* R.R. Mill. allied to *N. scrophulariaeflora* (Pennell) Hong showing similar morphology but distinguished by smaller habit and unique anther shape (Miller 2001). Taxonomic status of *Neopicrorhiza* remained uncertain and based on phylogenetic analysis of DNA sequence data it was concluded that *P. kurrooa* and *N. scrophulariflora* are close sister taxa and cannot be referred to separate genera. (Brummitt, 1992, Hong & al., 1998; Press & al., 2000; Mill, 2000, 2001; Polunin & Stainton, 1985; Tank & al., 2006; Uniyal & al., 2007; Chowdhery & al., 2008; Albach & al., 2005). Therefore it was suggested to merge the two genera (*Picrorhiza* and *Neopicrorhiza*) under *Picrorhiza* (Albach & al., 2005). Most recently, Pusalkar (2013) described fourth species in the group, *P. tungnathii* from western Himalaya. An analysis of literature revealed that, the distribution of *P. kurrooa* limited to western Himalaya (Pakistan, Jammu & Kashmir, Himachal Pradesh, Uttarakhand); *P. scrophulariaeflora* Pannell restricted in eastern Himalaya (China, Nepal, Bhutan, parts of Uttrakhand, Himachal Pradesh, Sikkim), *P. minima* Mill is endemic to Bhutan, while *P. tungnathii* Pusalkar is endemic to Indian western Himalaya (Uttarakhand and Himachal Pradesh).

All three *Picrorhiza* species are locally known in the western Himalaya as '*Kutki*, *Kedar kutki* and *Kadwi*', being one of the most important medicinal herb groups with high exploitation for trade. This medicinal herb group has recently been taken as high priority group for conservation and cultivation to meet commercial requirements and to avoid heavy depletion in the wild. Therefore, there is a need to authentication of this group of plants, at its taxonomic, phytochemicals and molecular level for scientific validation. In the present communication an attempt was made to differentiate these three species of *Picrorhiza* to namely *P. kurrooa* Royle ex Benth. *P. scrophulariiflora* Pennell, and *P. tungnathii* Pusalkar based on their taxonomic, ecological and phytochemical characters. We hope this will facilitate use of correct species in herbal industry avoiding high scale adulterants and also increase the efficacy in the Ayurvedic system of medicine.

MATERIALS AND METHODS

The plant samples were collected from Uttarakhand and Himachal Pradesh. All the specimens were deposited in the institution herbarium. For authentication, the herbaria CAL, BSD and DD were consulted along with relevant literature (Duthie 1906; Pennell 1943; Stewart 1972; Chowdhery & Wadhwa, 1984; Naithani 1985; Polunin & Stainton, 1985; Gupta & al., 1990; Aswal & Mehrotra, 1994; Hajra & Balodi, 1995; Gupta & Chauhan, 1998; Wu & Raven, 1998; Dhaliwal & Sharma, 1999; Singh & Rawat, 2000; Uniyal & al., 2007; Chowdhery & al., 2008; Chandrasekar & Srivastava, 2009).

DNA fingerprinting was done to study the genetic diversity using ISSR (Inter-Simple Sequence Repeats) technique between various samples collected from different geographical locations. DNA was isolated using Qiagen DNeasy plant DNA isolation mini kit (Qiagen, USA) and stored at -20°C. PCR was performed using 40 primers synthesized by Sigma Aldrich, Bangalore; in a reaction volume of 50µl using Taq PCR master mix (Qiagen USA). Fragments were separated on basis of size using Agarose gel Electrophoresis. Gels were visualized with AlphaImager EC imaging system (Protein Simple, CA, USA) and analysis of ISSR bands were performed by Alphaview software version 3.4. The presence of an amplified product (band) in each position was recorded as 1 and absence as 0. Fragment size of each band in bp (base pairs) was calculated using GeneRuler 100bp DNA Ladder (Thermo Scientific). Banding patterns produced by different accessions were compared to determine similarity or dissimilarity. Dice coefficient of similarity was measured and a dendrogram based on similarity coefficients was generated by using Unweighted Pair Group Method with arithmetic Mean (UPGMA). The computer package NTSYSpc 2.2 (Rohlf 1990) was used for cluster analysis.

RESULTS

Diagnostic Characters: *P. scrophulariaeflora* Pennell is characterized by dwarf habit; zygomorphic, 2-lipped (*Scrophularia* -like) corolla exceeding calyx; corolla lobes unequal, externally glandular-pubescent; upper corolla lobe 2-lobed at apex; stamens didynamous, not or slightly exserted from the corolla and pollen of the *Paederota* -type (**Fig. 1**).

P. kurrooa is characterized by comparatively taller habit; densely (25–)30–50-flowered spike; actinomorphic or sub-actinomorphic corolla much smaller than and concealed within large calyx; inconspicuous, straight corolla tube much smaller than corolla lobes; corolla lobes equal, similar, ovate or oblong-ovate, externally glabrous, densely ciliate along margins, non-galeate, acute, undivided at apex, pilose inside, stamens equal (not didynamous) with pollen of the *Scrofella* -type, and style 5–10 times as long as the corolla **(Figs. 2,4)**.

P. tungnathii Pusalkar is distinguished from of the allied *P. kurrooa* Royle by being 10-25 cm tall, having

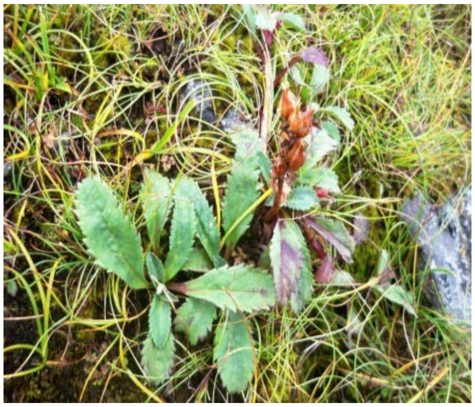

Fig. 1: *Picrorhiza scrophulariiflora*

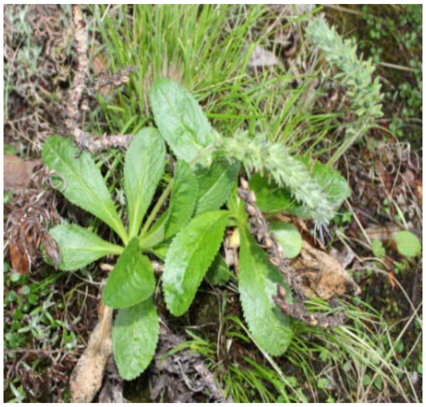

Fig. 2: *Picrorhiza kurrooa*

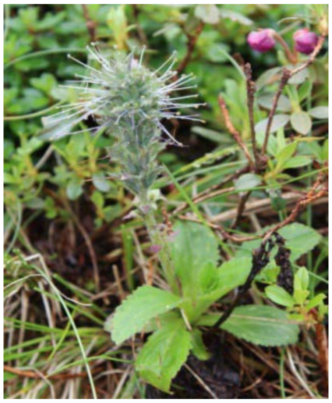

Fig. 3: *Picrorhiza tungnathii*

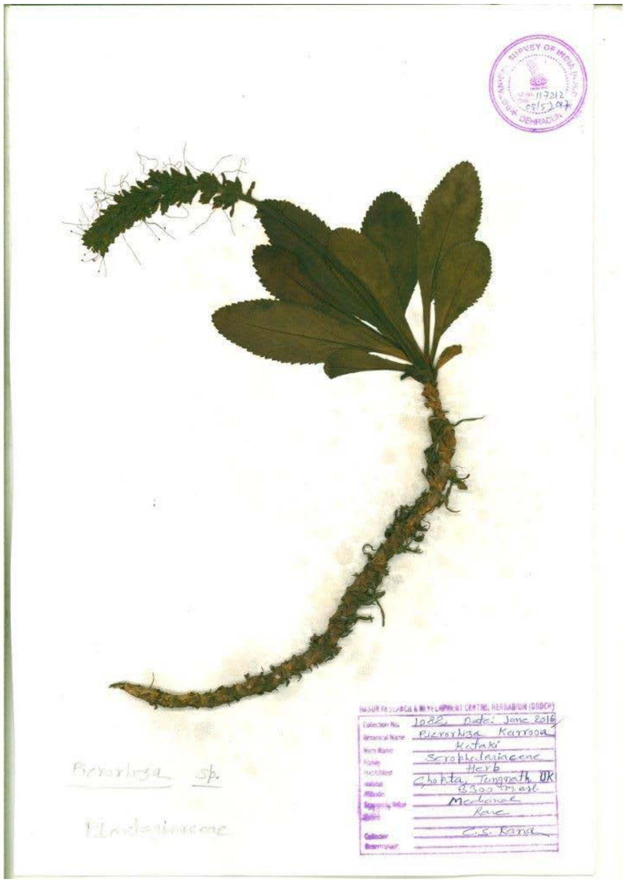

Fig. 4: *Picrorhiza kurrooa* Royle ex Benth.

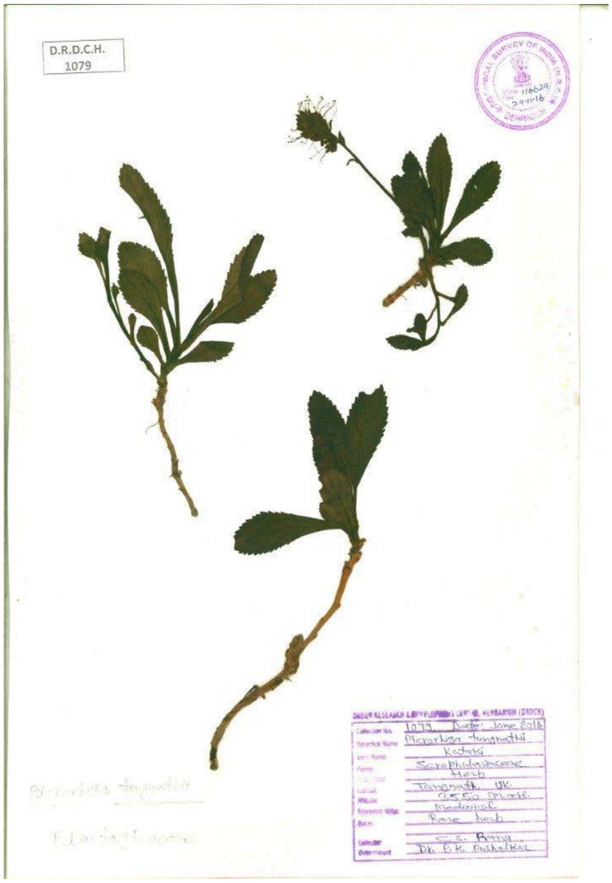

Fig. 5: *Picrorhiza tungnathii* Pusalkar

a moderately dense, (10–)15–25-flowered spike, a zygomorphic, 2-lipped, glandular-ciliate corolla that is equaling or slightly exceeding the calyx and partly visible or sub-exserted between the calyx lobes, a conspicuously long (longer than the corolla lobes), curved corolla tube (1/2–4/5 the length of the calyx), unequal corolla lobes that are ovate-lanceolate to lanceolate, acute to sub-acuminate and moderately ciliate, a mid-lobe of the upper lip that is obliquely erect, galeate with retuse or emarginate apex; lateral corolla lobes that are slightly smaller than the mid-lobe of the upper lip; a lower corolla lip that is slightly shorter than the lateral lobes, obliquely erect or spreading, didynamous, long-exserted stamens, pollen of the *Paederota* -type and a style that is 2–3 times as long as the corolla. (**Figs. 3,5**).

DNA Studies: Average similarity index observed between the studied 7 accessions suggests high genetic diversity between the studied samples. Dendrogram thus obtained indicated two major clusters. The first cluster was further divided into two sub groups with 69% similarity, separating one accession (PKTLS-1) from remaining five accessions. This clearly classifies the two sub-groups on basis of geographical location of collection (PKTLS-1 from Himachal Pradesh while other 5 members of the sub-groop are collected from Uttarakhand). The second cluster represented one accession (PKCHR-1) with a similarity of 36 % with the second cluster. (**Fig. 6**).

Distribution: *P. scrophulariiflora* Pennell, is widely distributed in Himalaya including Nepal and usually grows on open, stony and grassy slopes and on the turf of glacial flats (Dutta 2007). *P. kurrooa* is endemic to western Himalaya grows on moist, hanging stony walls and grassy sub-alpine slopes between 3000-3300 m. *P. tungnathii* Pusalkar grows in moist, rocky slopes as well as in organic soils in alpine Himalayas.

DISCUSSION

Picrorhiza/Kutki is one of the important herbal drugs with an annual demand of 4–6 tones in India (Olsen 1998; Arya & al., 2013). *P. kurrooa* Royle ex Benth. listed under the 'endangered' species category while both *Picrorhiza kurrooa* and *P. scrophuleriiflora* are also listed in the annexure -II of CITES (CITES 1973-2013).

Medicinal Uses: *Picrorhiza* species contain several bioactive compounds that have therapeutic properties.

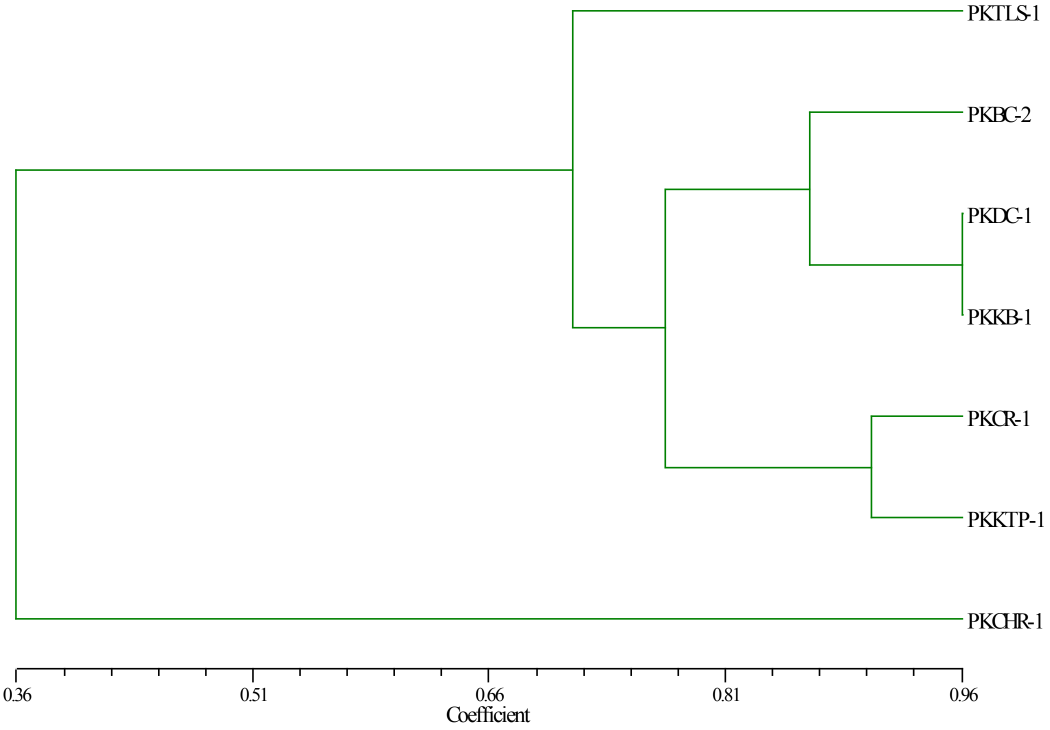

Fig. 6: Dendrogram (UPGMA) showing the Genetic relationship between the accessions of *Picrorhiza* Scale: Dice coefficient.

Both species are known under the same local name and used for similar purposes only chemical compositions vary. They are extensively used in traditional as well as modern medical systems of India, China, Tibet, Nepal and Sri Lanka (Hu & Yang, 2005; Tong & al., 2008). *P. kurrooa* is used for the treatment of liver disorders, fever, asthma and jaundice, gastrointestinal and urinary disorders, leukoderma, snake bite, scorpion sting and inflammatory, hepatoprotective, anti-inflammatory, immunomodulatory, free radical scavenging, gastric ulcer, anti-allergic and antianaphylactic activities, anti-hepatitis-B surface antigen activity (Kaphai & al., 1993, Gaikwad & al., 2011). The active constituents Kutkiol, Kutkisterol, D-Mannitol, Apocyanin: phenol glucosides; androsim and picein iridoid glycosides; Kutkin, Picroside I, II & III; Kutkoside, Minecoside, Picrorhizin, Arvenin are obtained from *Picrorhiza kurrooa* (Shah & Vashney, 2013). While, *Picrorhiza scrophulariflora* is used in traditional Chinese medicine also as antidiabetic, antiasthmatic, cardioprotective, anti-healing, antioxidant and antiradical activities, antiulcer and anticancer activity, a selective enhancer of neuron growth (Hu & Yang, 2005; Tong & al., 2008; Yin & al., 2010).

Adulterants: Adulteration either intentional or unintentional reduces potency of drugs. Taking due advantage of the trade name *Kutki*, the rhizome of *Picrorhiza* are being adulterated with inter and intra genus.

CONCLUSION

In the present communication, the genetic diversity using DNA fingerprinting wherein high variations are found within the studied samples, suggesting presence of multiple species. Therefore, all populations of *P. kurrooa* and *P. scrophulariflora* are required to be reassessed to ascertain the exact population status. The prime requirement is the to use of correct species for efficacy of herbal industry and avoids any sort of adulterants. Inaccurate identification of different species of Picrorhiza is one of the main reason for adulteration.

Phytochemistry of *P. kurrooa* Royle ex Benth., and *P. scrophuleriiflora* Pannell have been studied extensively, whilst *P. tungnathii* Pusalkar is yet not studied. There is no report after inclusion and discovery of *P. tungnathii*. In view of a recent delimitation of these species, the possibility of currently available chemical analysis results for *P. kurrooa* may belong to mixed samples (if done based on material available in markets) and hence further studies are needed to isolate biologically active compounds and get clear idea of correct chemical spectrum for each species.

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